Supplemental Table 1: Relative growth of untreated and treated cultures

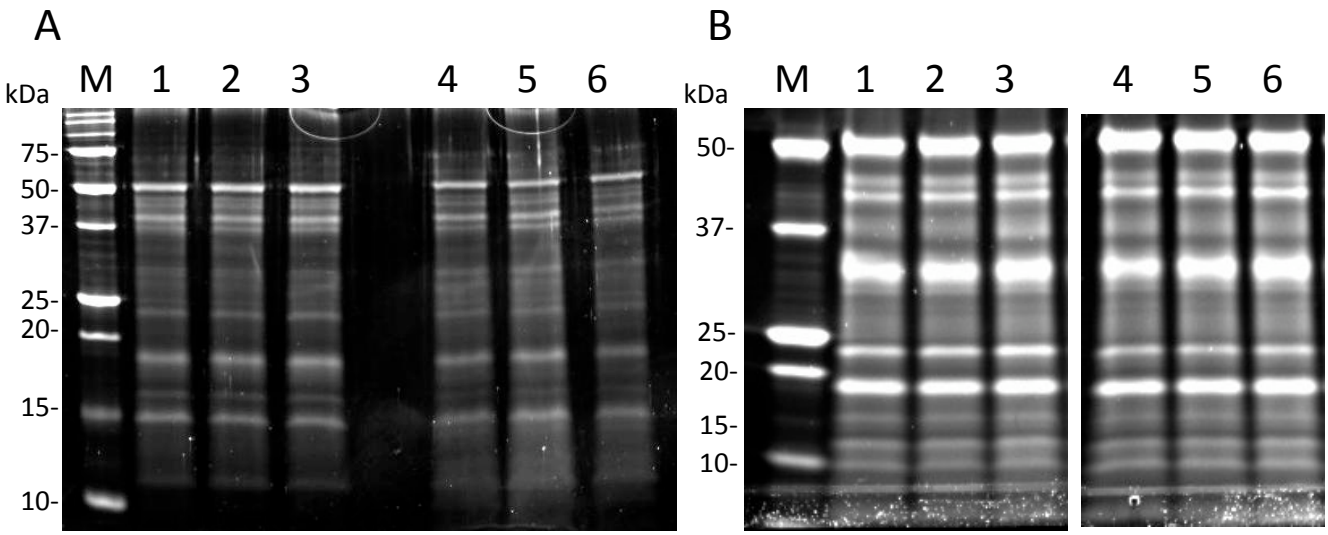
Strain	Treatment [a]	Untreated (Avg. CFU x 10^8 ± STD)[b]	Treated (Avg. CFU x 10^8 ± STD)	P-value[c]
PA14	H_2O_2	6.99 ± 2.16	3.71 ± 0.55	0.11
PA14	D-cycloserine	0.52 ± 0.12	0.33 ± 0.33	0.24
PA14	polymyxin B	6.2 ± 1.72	2.72 ± 1.49	0.006
PA14	25°C to 37°C shift	10.56 ± 2.07 (25°C)	7.97 ± 1.91 (37°C)	0.08
PA14	25°C to 39°C shift	10.56 ± 2.07 (25°C)	6.18 ± 4.02 (39°C)	0.07
PA14 *ΔalgU*	H_2O_2	4.82 ± 0.65	3.73 ± 1.01	0.20
PA14 *ΔpqsA*	D-cycloserine	10.18 ± 3.01	8.9 ± 1.42	0.44
PA14 *ΔpqsA*	polymyxin B	10.18 ± 3.01	6.98 ± 0.26	0.076
PA14 *ΔpqsA*	H_2O_2	10.18 ± 3.01	9.23 ± 0.71	0.53
PA14 *ΔoxyR*	H_2O_2	5.57 ± 0.74	6.9 ± 2.05	0.22
PA14 $+H_2O_2$ + IPTG	MucD overexpression (pLW112 *vs.* pMF54)	4.85 ± 0.37	2.4 ± 0.25	0.001

[a] Treatment conditions are detailed in the experimental methods.

[b] CFU were obtained by dilution plating; Standard deviation for the CFU average from individual replicates is noted (STD)

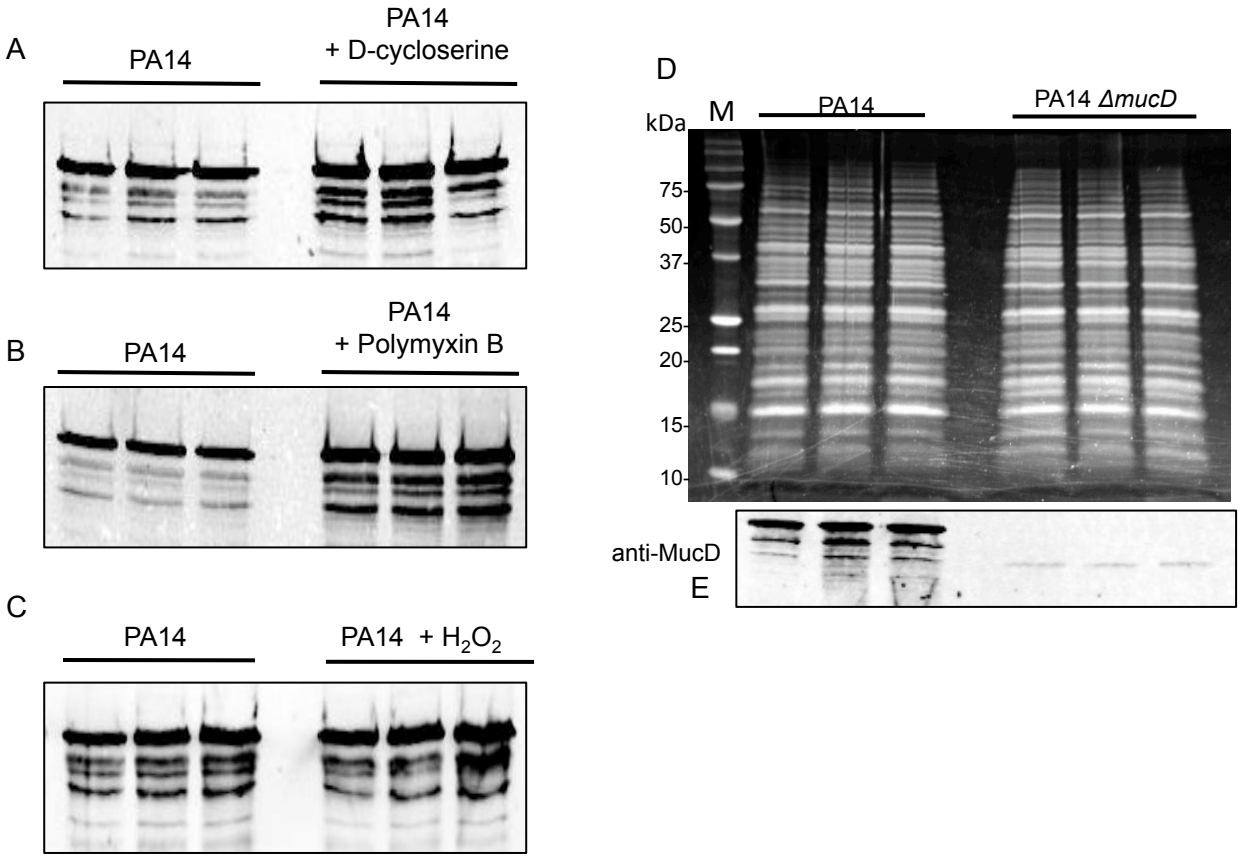
[c] P-value denotes level of significance calculated by Student's T test between untreated and treated average CFU.

Fig S1



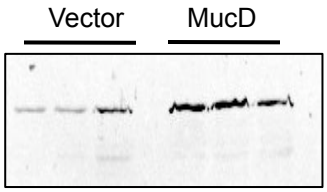
Supplemental Figure 1 – SDS-PAGE of OMV protein profiles with significant CFU differences to check for cell lysis. All samples were loaded with 1µg total protein. A) PA14 was treated with or without 4µg/ml polymyxin B and OMVs were purified. OMVs were run on 15% SDS-PAGE. Lane 1-3 are three replicates of untreated PA14 OMVs. Lanes 4-6 are three replicates of treated PA14 + PmxB OMVs. B) PA14 pMF54 and PA14 pMF54/MucD were treated with hydrogen peroxide and had their OMV production determined. OMVs were run in a 4-20% gradient gel. Lane 1-3 are three replicates of PA14 pMF54 + H_2O_2 OMVs. Lanes 4-6 are three replicates of treated PA14 pMF54/MucD + H_2O_2 OMVs. Migration of protein molecular weight standards (M) are indicated (in kDa).

Fig S2



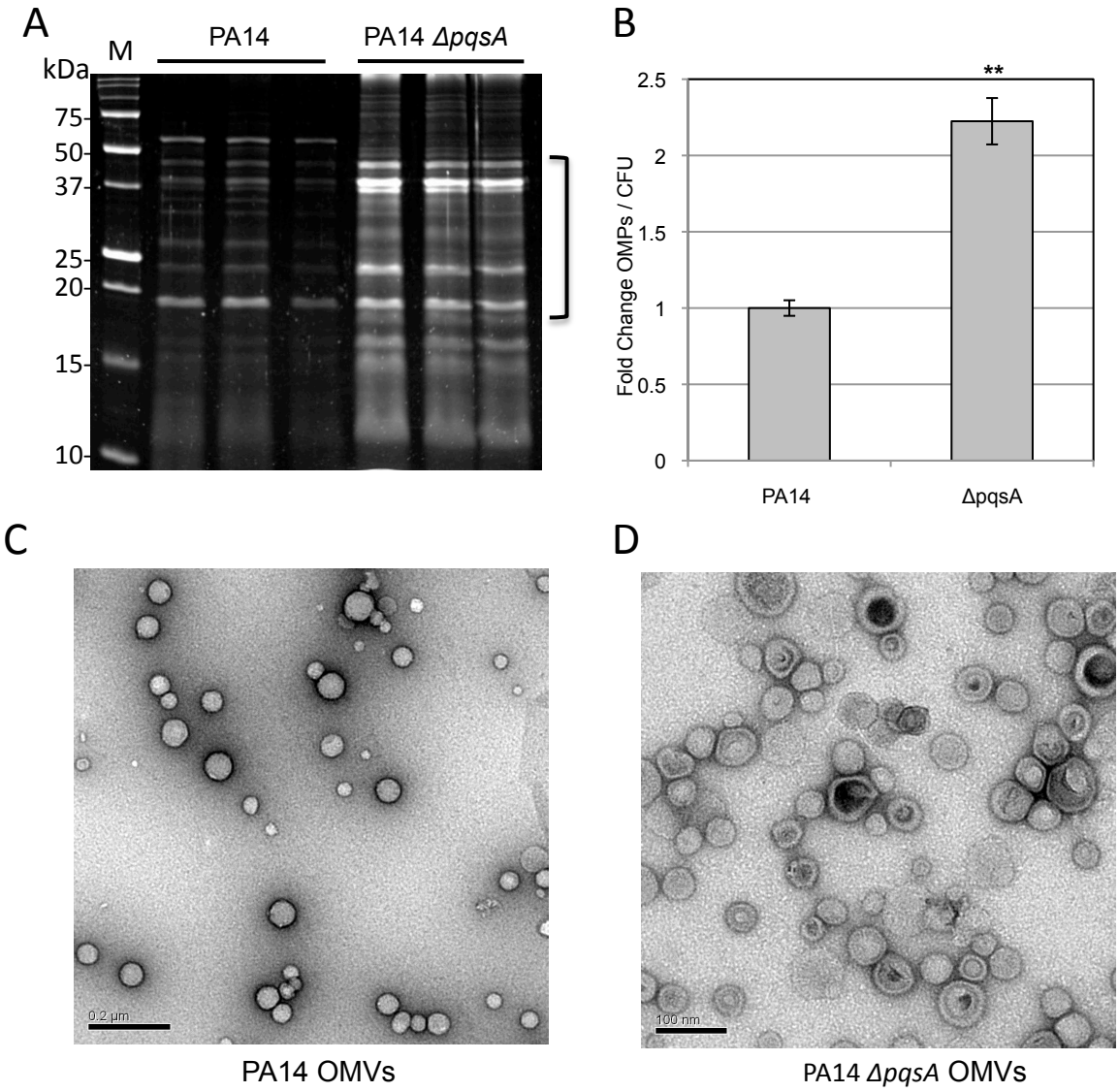
Supplemental Figure 2- Stress induced periplasmic MucD levels of PA14. Anti-MucD western blot of three biological replicates of PA14 pLW127 cultures treated without or with 250 µg/ mL D-cycloserine (A) without or with 4 µg/ mL polymyxin (B), and without or with 250 µM hydrogen peroxide (H_2O_2)(C). 4 µg (A and B) and 10 µg (C) total periplasmic protein was loaded per lane. (D) PA14 and PA14 *ΔmucD* were grown to late-log phase and periplasmic contents were isolated. 2 µg total protein was loaded and run on SDS-PAGE followed by Sypro Ruby stain. Migration of protein molecular weight standards (M) are indicated (in kDa). (E) Anti-MucD immunoblot of the above gel indicating the multiple cleavage forms of MucD. Due to multiple cleavage forms being present, the total lane was quantified as total periplasmic MucD.

Fig S3



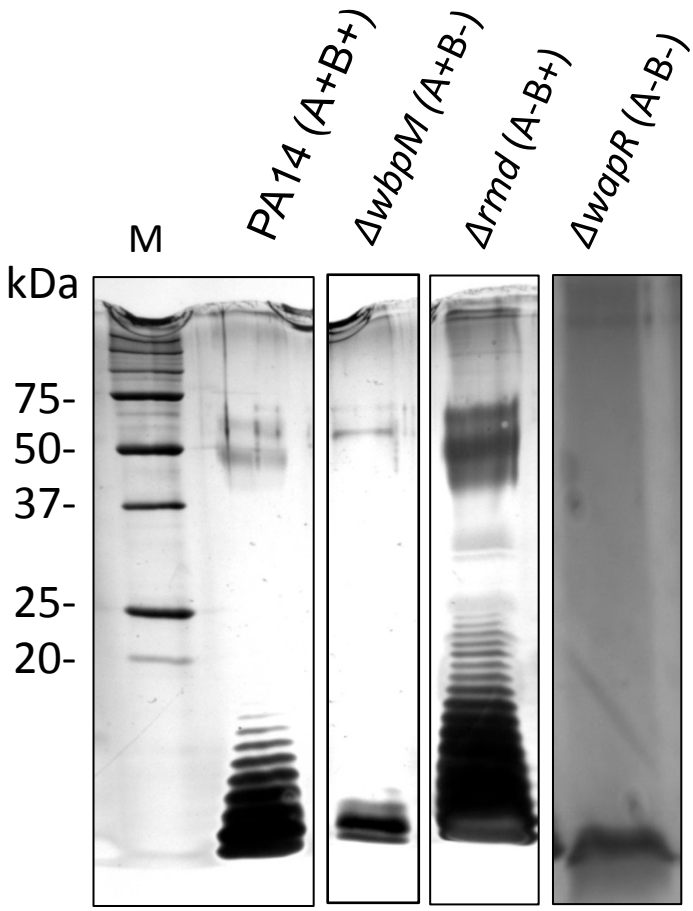
Supplemental Figure 3- Periplasmic MucD levels upon induced MucD overexpression. Anti-MucD western blot of three biological replicates of PA14 pLW112 cultures induced without or with 1 mM IPTG. (5 µg total protein was added per lane)

Fig S4



Supplemental Figure 4 – Quantitation and imaging of PA14 and PA14 Δ*pqsA* OMV production. (A) SDS-PAGE of equal volumes of 3 biological replicate preparations of density gradient purified PA14 and PA14 Δ*pqsA* OMVs. Migration of protein molecular weight standards (M) are indicated (in kDa). (B) Protein bands in part A (bracketed) were quantitated by densitometry and normalized by CFU in the culture at the time of harvest and divided by the OMV yield of PA14. Electron micrographs of negatively stained PA14 (C) and PA14 Δ*pqsA* (D) OMVs purified by density gradient from log-phase growth cultures (images taken by A. Manning). Size bar: 200 nm (C), 100 nm (D).

Fig S5



Supplemental Figure 5–LPS analysis. LPS (PA14, PA14 Δ*wbpM,* PA14 Δ*rmd* , PA14 Δ*wapR)* was isolated by organic extraction and separated using15% SDS-PAGE. The gels were silver stained to resolve LPS banding patterns. Migration of protein molecular weight standards (M) are indicated (in kDa).